

FORTNIGHTLY MOLTING AND REPRODUCTIVE CYCLES IN THE SAND-BEACH ISOPOD, *EXCIROLANA CHILTONI*

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The degree to which environmental variations affect selection for temporally organized activities is related to both the amplitude and predictability of the environmental cycles. The ecologist should expect to find well-developed temporal organization in the activities of species inhabiting highly variable but predictable environments (Enright, 1970). The wave-washed sand-beach habitat epitomizes this kind of situation. The unique characteristics of the sand-beach habitat, especially the steep physical gradients, which vary over a broad range depending on the stage of the tide and time of day, have led to a considerable interest in the ecology and behavior of its fauna. At any point on the beach, many aspects of the physical environment including wave turbulence, consistency of the substrate, temperature, salinity, pH, and the concentrations of oxygen and carbon dioxide (Bruce, 1928; Emery and Foster, 1948) show marked variations with time. Only a limited number of species of macrofauna have evolved the ability to tolerate these varying physical conditions.

The behavior of the sand-beach isopod, *Excirolana chiltoni*, is to a great degree governed by the tides. When the advancing wave wash of the incoming tide reaches the level on the high intertidal beach where these isopods are buried, they leave the sand to swim and forage in the wash zone. This period of swimming activity continues for approximately 2 hours past tide crest, after which the isopods once again burrow into the sand, which allows them to escape the ebbing tide and re-establish their position on the exposed beach until the next period of high water.

The survival value of temporally organized activities is particularly evident for this species, since its distributional zone lies farther shoreward than that of most intertidal sand-beach crustacea (Enright, 1961; Clark, 1969). *Excirolana chiltoni* is essentially a marine animal, requiring for its survival the periodic flooding of its habitat during high tide. These animals must maintain their distribution in an area of the beach where environmental gradients are steep and where an error in positioning of a few meters in the landward direction could place an individual out of the range of physically tolerable conditions. Since an individual could be incapacitated while molting, the question arises as to how this important activity might be coordinated to the tide cycle in a manner which allowed this species to maintain a favorable position in its habitat. This requirement provides the focal point for the present study.

Excirolana chiltoni stores calcium salts in localized concretions in its integument prior to ecdysis. The examination of preliminary collections of *Excirolana* taken from Scripps Beach (La Jolla, California) at intervals of 3 to 4 days during the summer of 1969, indicated that in some samples individuals with well-developed

concretions were quite common while in other samples such individuals were either rare or entirely absent. Since the degree of development of these concretions parallels the molt cycle, this observation suggested that isopods tend to molt synchronously in the field. A more intensive and systematic program was undertaken in the summer of 1970 to look at the possibility of synchronous molting in greater detail, the results of which are presented here. In addition to the field study, molting frequency was monitored in a laboratory population held under non-tidal conditions in order to evaluate the possibility of an endogenous mechanism controlling the molt cycle.

METHODS

A 10 meter stretch of beach fronting on the Scripps Institution of Oceanography (La Jolla, California) was chosen as the study area. This beach is exposed to the open sea and has an average slope of 1–2% and a median sand grain diameter of 0.20 mm.

The backshore is eliminated by the presence of a concrete seawall; this makes Scripps Beach only slightly atypical, since the backshore is not well developed in adjacent areas where sandstone cliffs begin a few meters landward of the high-tide wash line. Wave wash reaches the seawall on the highest tides of the month, at least during the winter, when the beach is at its lowest level due to erosion by heavy surf. Wave wash rarely reaches the seawall during the summer when the beach is 50 to 100 cm higher as the result of sand deposition during periods of low wave action.

The tidal range on Scripps Beach at the time of new and full moon (spring tides) is considerably greater than the range at the first and last quarter (neap tides). The mean tide range is 3.6 feet but differences between high and low water can be as much as 6–8 feet during spring tides. During the lowest low tides, the exposed beach extends 150–200 meters from the sea wall, while during the highest low tides 50 meters or less of the beach may be exposed.

Excirolana chiltoni was collected from Scripps beach at 2-day intervals from June 17 to July 17 during the summer of 1970. On each sampling day a 50 meter transect line was laid out perpendicular to the seawall along the beach profile and a continuous trench 25 cm wide and 10 cm deep was dug parallel to this reference line. The trench, consisting of a series of contiguous 3-meter long sections, was sufficiently long to encompass the entire zonal range of the isopod band for that day. Sand samples from each 3-meter long section of the transect were sieved through a 0.5 mm mesh which retained even the smallest individuals in the population. Isopods collected in this manner were immediately killed with isopropyl alcohol and brought into the laboratory where they were preserved in buffered 5% formalin seawater.

RESULTS

Distribution

Figure 1 indicates changes in the distribution of *Excirolana* which were observed during the study period. The upper limit of the zonal band corresponds with the position of the washline of the preceding high tides on those occasions

when the position of the high tide waterline was recorded (July 5 through July 17). The most obvious feature of these distributions is a movement of the population to progressively higher levels on the beach during weeks prior to both new and full moon (as the spring tides approached) and to lower levels on the beach during weeks following new and full moon (as the neap tides approached).

The position of the zonal band appears to have retained the same position relative to the high tide washline as the washline changed during the spring-neap cycle, resulting in a fortnightly shift in zonation. The amplitude of this movement is, however, somewhat different in the two cycles shown in Figure 1, although

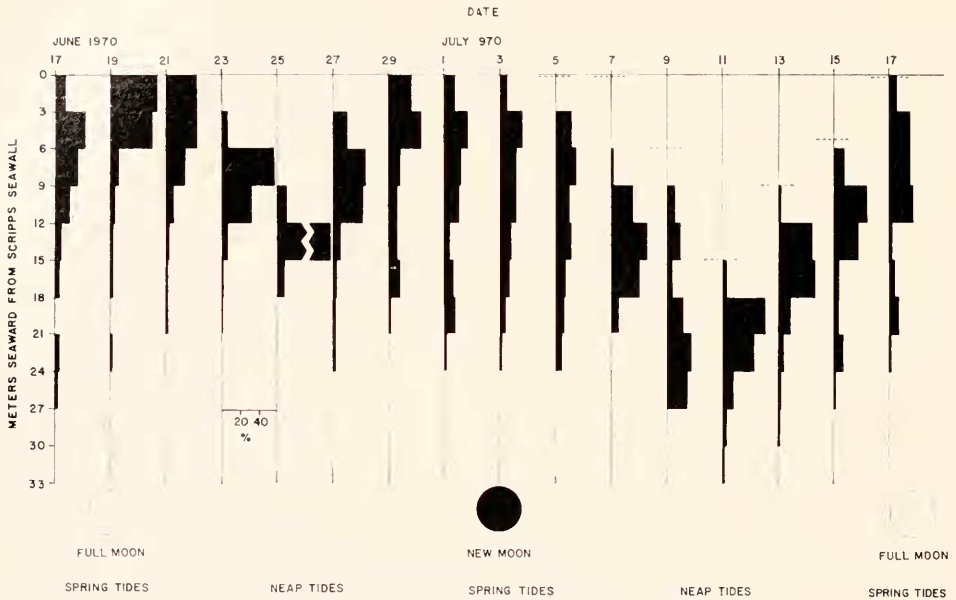


FIGURE 1. Semimonthly changes in the position of the *Excirolana* zone. The relative abundance of isopods in 3 meter sections of a continuous 25 cm wide transect across the zonal range, is indicated by each histogram. Transects were taken at two-day intervals on Scripps Beach. The width of the black bars indicates the relative abundance of isopods in each of the 3 meter long sections of each transect, expressed as a percentage of the total population. The dashed lines indicate the level of the beach reached by high tides during the interval between consecutive transects. This level was determined on July 5th to July 17th by observing the position on the beach where the previous trench had been obscured by wave wash.

tide heights on corresponding days in each cycle were similar. This appears to be the result of the truncation of the distribution during the first cycle (June 19 to July 3) by the presence of Scripps seawall. The wave wash on spring tides struck the seawall which effectively stopped any further shoreward movement. This is somewhat atypical for this season, since in most years the level of the beach is sufficiently high by June to prevent wave wash from reaching the seawall even on the highest tides. An unusual period of high storms in the previous winter had eroded the beach to the extent that it had not yet reached its typical summer level by June 1970. The level of the beach, however, rose approximately

$\frac{1}{2}$ meter from July 1 to July 9, which resulted in a seaward displacement of the distribution during the latter half of this study.

Similar fortnightly movements up and down the beach are a conspicuous feature of the distribution of a number of sand-beach animals, having been demonstrated for the bivalve *Donax gouldi* (Loren Haury, Scripps Institution of Oceanography, unpublished), and the cirrolanid isopod *Eurydice pulchra* (Fish, 1970). They probably occur as well in the amphipod, *Synchelidium* sp. (Enright, 1961) and the anomuran crab *Emerita analoga* (Efford, 1965). While only the daily tidal component of migration has been looked at in the last two species, each assumes a zonal distribution on the exposed beach during low tide which is correlated with the amplitude of the previous high tide. A fortnightly migration is therefore implied, although time-series data over the spring-neap cycle were not actually collected.

Calcium storage in the integument

Two pairs of previously undescribed structures which store calcium carbonate and phosphate salts are present in *Excirolana chiltoni* (Figure 2). They are

DERMOLITHS OF *Excirolana chiltoni*

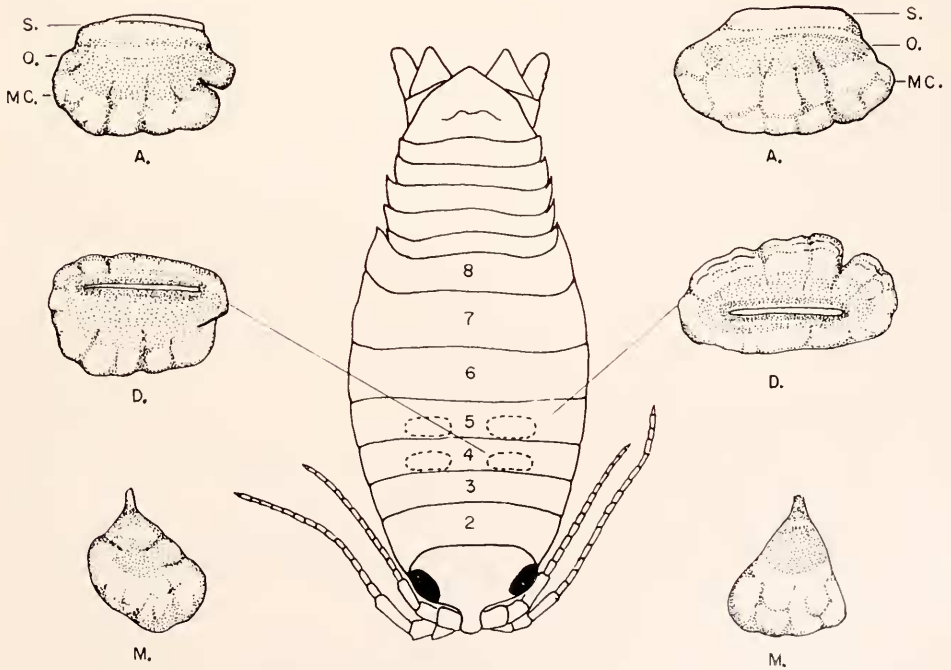


FIGURE 2. Sites of mineral storage (dermoliths) in the integument of *Excirolana chiltoni*: these structures can be seen through the translucent dorsal body wall: A., anterior view; D., dorsal view; M., median view; S., stalk area; O., opaque central core; MC., molded area of calcium salts.

attached to and continuous with the anterior margins of the 4th and 5th thoracic segments along the dorsal surface. While they serve the same function as the gastroliths of the decapods, they differ in that there are two pairs rather than one, and that both are apparently derived from the tissues of the integument rather than the epithelium of the foregut. The storage structures, because they are derived from the mineralized layer of the integument, will be referred to as dermoliths.

Each dermolith is composed of three morphologically distinguishable parts. These are: (1) a broad dorsal stalk composed of material which is continuous with the mineralized layer of the integument, connecting to (2) an opaque central core, which is surrounded by (3) a large sculptured mass of calcium salts. The size of the dermoliths increases markedly prior to the molt, indicating that their primary function is the storage of mineral reserves to be used in hardening the post-ecdysial exoskeleton. There is a consistent difference in the shape of the anterior and posterior pairs of dermoliths. Dermoloths of the anterior set possess a prominent lateral projection while those of the posterior pair have a deep cleft on their trailing margins.

The dermoliths dissolve in dilute hydrochloric acid, except for the opaque central core which leaves a small residue. They produce gaseous bubbles, indicating the presence of calcium carbonate. X-ray diffraction patterns of material from 20 dermoliths failed to show any of the characteristic peaks of the known crystalline forms of calcium carbonate (calcite, aragonite or vaterite). A rather low diffuse peak was present, diffracting at an angle of about 30° which is characteristic of phosphate minerals. Calcium carbonate must, therefore, be present in an amorphous form. Prenant (1927) has found that only a few per cent of phosphate ions on a molar basis with calcium ions are sufficient to inhibit the crystallization of calcium carbonate; x-ray fluorometry indicates significant quantities of phosphorous in addition to calcium in the dermolith of *Excirolana* (Klapow, 1971). The mineral contents of fully developed dermoliths can be as much as 37.8% of the mineral salts in the entire exoskeleton.

Molting in *Excirolana chiltoni* takes place in two stages. After the dermoliths have reached their maximum size, a split develops in the exoskeleton between the 5th and 6th thoracic somites. The exoskeleton posterior to the split (the last 3 thoracic somites and abdomen) is then cast. The anterior exoskeleton is not cast immediately but follows approximately 25 hours later. During this intervening period, the new exoskeleton on the posterior part of the isopod expands while the anterior portion of the isopod, still confined by the pre-molt exoskeleton, retains its original dimensions. An individual at this stage can be recognized by the break in profile at the junction of the 5th and 6th thoracic somities: the profile of the ventral lateral margin of the thoracic somites is straight during the intermolt but shows a distinct step-like expansion at the 6th thoracic segment once the posterior exoskeleton is cast.

During this half-molted stage, the mineral reserves in the dermoliths are absorbed into the blood, and the isopod assumes an opaque white aspect. At the same time that the dermoliths are being absorbed, re-calcification of the newly formed and fully expanded posterior exoskeleton is in progress (acidifying the posterior exoskeleton during the half-molted stage leads to the evolution of gaseous

bubbles, indicating the presence of CaCO_3). Absorption and deposition occur simultaneously in different portions of the integument. This raises an interesting physiological question since these processes are generally considered to be under the control of blood-borne hormones which have a general effect throughout the body (Guyselmann, 1953). The manner in which a widely distributed blood-borne substance produces a different response in separate parts of the integument remains an open question.

Following the absorption of the dermoliths, the anterior exoskeleton (head and first 5 thoracic somites) is shed and the isopod returns to its normal intermolt proportions. *Excirolana* retains its opaque white aspect until the remaining calcium salts, dissolved in the blood, are deposited in the anterior integument. On occasion, small portions of the dermoliths may not be completely absorbed and can be recognized on the exuvia of the anterior cast, demonstrating clearly that they are, in fact, derived from the integument.

A two stage molting process with a considerable delay between posterior and anterior casts has been reported in *Ligia* (Tait, 1917; Numanoi, 1934) *Oniscus* (Messner, 1966) and *Porcellio* (Messner, 1966). All of these isopod genera belong to the suborder Oniscoidea. *Excirolana* belongs to the suborder Flabellifera. The occurrence of this peculiar two-phase molt in two distinct suborders suggests that it may very well be characteristic of all the Isopoda. Schultz (1969) thought that this was, in fact, a general feature of molting in isopods, although he was undoubtedly mistaken when he stated that the anterior exoskeleton is shed first. In all of the cases cited above, the posterior portion of the exoskeleton is shed first.

Fortnightly molting and reproductive cycles: field data

The series of 25 cm wide continuous transects yielded on the average 293 isopods per collection (range 67–540 for 16 collections). Each specimen was examined and the following information recorded: (1) Total length, measured from the tip of the telson to the most anterior point on the head; (2) Stage of development; juveniles were classified as either 1st, 2nd, or 3rd stage manca; older individuals were sexed; (3) Stage of the molt cycle as characterized by the development of the dermoliths; (4) The presence or absence of food in the gut; (5) The presence or absence of external parasites on the ventral thorax.

There are 3 manca stages in *Excirolana chiltoni*. The 1st and 2nd manca lack the 8th pair of thoracic legs, which appear in a rudimentary condition in the 3rd manca stage and are fully developed in the post-manca stages. The 1st and 2nd manca stages which are morphologically similar were separated by size: specimens shorter than 2.5 mm were considered to be 1st stage while those that were longer than 2.5 mm were designated as 2nd stage. The size frequency distributions of 1st and 2nd stage manca overlap somewhat, although not to the extent where the selection of the 2.5 mm cutoff point would result in a serious error. The half-molt, in transition from the 1st to 2nd manca stage is morphologically unique. It is the only half-molt stage which lacks any trace of the 8th pair of thoracic legs.

The dermoliths are not always present but undergo a cycle of accretion and absorption which parallels the molt. Their stage of development can, therefore,

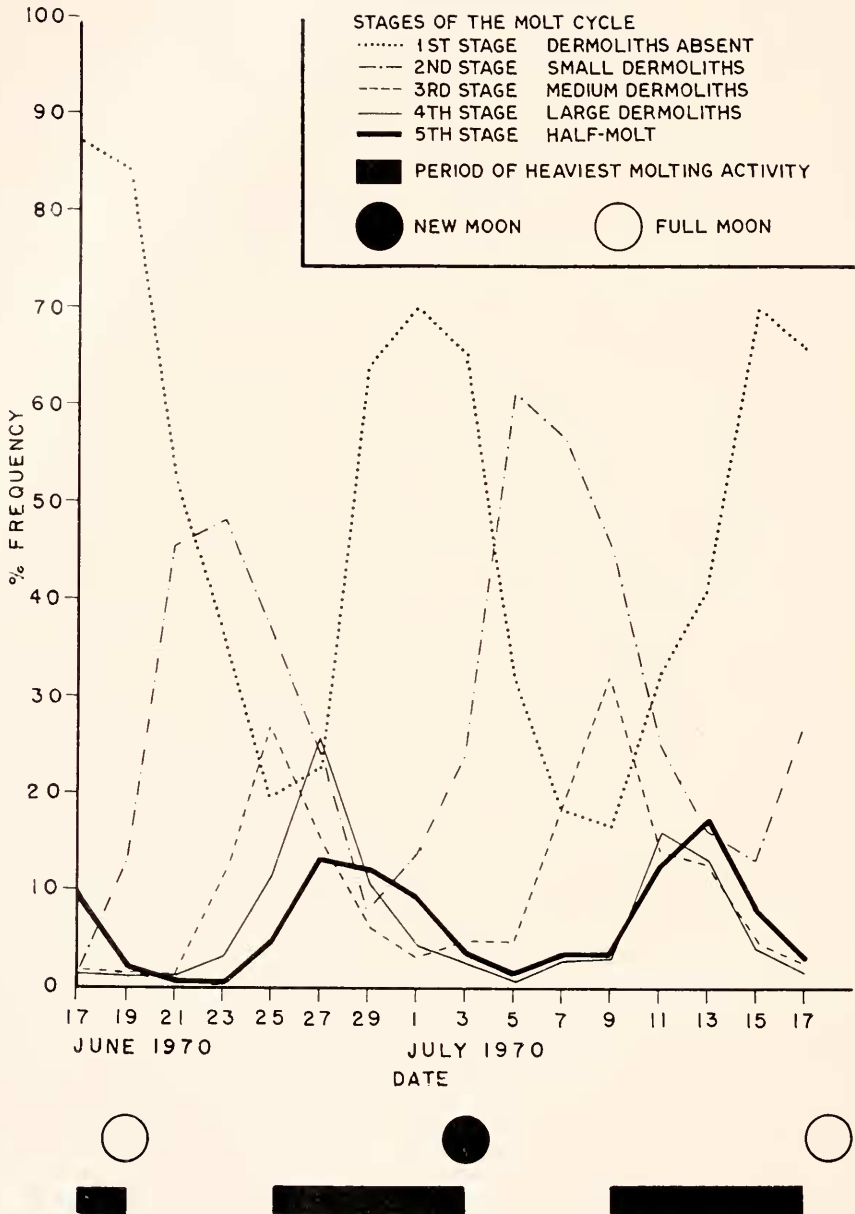


FIGURE 3. Relative abundance of the 5 stages of the molt cycle as a function of time. The half-molt stage represents isopods which are in the process of molting.

provide convenient indices for the molt cycle. The molt cycle was divided into 5 stages based on this character. The 1st intermolt stage lacks dermoliths entirely. This is followed by the 2nd stage (small dermolith stage) in which the broad stalk area of the dermolith is present. In stage 3, both stalk area and opaque central core are present (medium dermolith stage), while in stage 4 the dermoliths are fully developed with stalk area, opaque central core and the large molded area of calcium salts all present. The 5th stage is the half-molt individual which has shed the posterior exoskeleton, and is the stage in which the dermoliths are in the process of being absorbed. Previous schemes to classify the molt cycle of crustaceans have been based on histological changes in the integument (Travis, 1955, 1957). The important advantage in defining the molt cycle of *Excirolana* by the development of dermoliths is that this character can be assessed by direct examination, since the dermoliths are clearly visible through the translucent dorsal body wall in most specimens. Large opaque individuals were dissected to determine the degree of development of their dermoliths.

The presence or absence of external parasites on the ventral body surface was also recorded. These parasites are stalked ciliate protozoans which attach to the integument and are lost with each molt. Isopods which have just cast are free of parasites but become reinfested with time. The degree of infestation of the population can therefore be used as an indication of the time since the majority of individuals last molted.

Gravid females do not molt while they are carrying embryos (gestation lasts for approximately 2 to 3 months in *Excirolana chiltoni*). For this reason they are not included in the analysis of molting frequency in the field. Only 71 gravid females were found out of 4690 specimens examined. This constitutes only 1.5% of all collections so that their exclusion from the analysis is relatively unimportant.

Figure 3 shows the relative abundance of each of the 5 stages of the molt cycle as they vary with time. Note that there is a fortnightly variation in the relative abundance of each stage. Note also that there is a progression in the abundance of the stages; first the curve indicating the relative abundance of isopods without dermoliths peaks (June 17), followed a few days later by a peak in the abundance of isopods with small dermoliths (June 23), then medium dermoliths (June 25), then large dermoliths (June 27), and finally the half-molt stage. The same progression in the abundance of stages was repeated in the second half of the study so that two complete molt cycles are indicated during the month of field collections. These two facts, the fortnightly variation within each stage and the ordered progression in peaks of abundance of the 5 stages, indicate that molting in *Excirolana chiltoni* is synchronized to a fortnightly cycle. Most of the actual molting activity is confined to a week prior to either the new or full moon, as indicated by the higher relative abundance of half-molted isopods at these times. Peak molting activity occurs in the middle of these intervals, that is, 4 to 5 days before the spring tides.

The numbers of cast exoskeletons found in the field collections show a fortnightly periodicity as well. Of the 102 intact exuvia (either anterior or posterior casts) which were found in the field collections, 94 were recovered during the weeks prior to new or full moon while only 8 were found on the weeks following new or full moon.

Figure 4 provides additional evidence for a synchronized fortnightly molting cycle. The dashed curve represents the percentage of parasitized isopods in the population as a function of time. Note that the incidence of parasitism decreases as the population enters the period of heaviest molting activity and rises sharply during the weeks following the new or full moon, as individuals become re-infested with external parasites following the molt.

The percentage of isopods with food in their guts, indicated by the solid line in Figure 4 also shows a fortnightly variation. Two alternative hypotheses could account for this observation: there may be a fortnightly variation in the availability

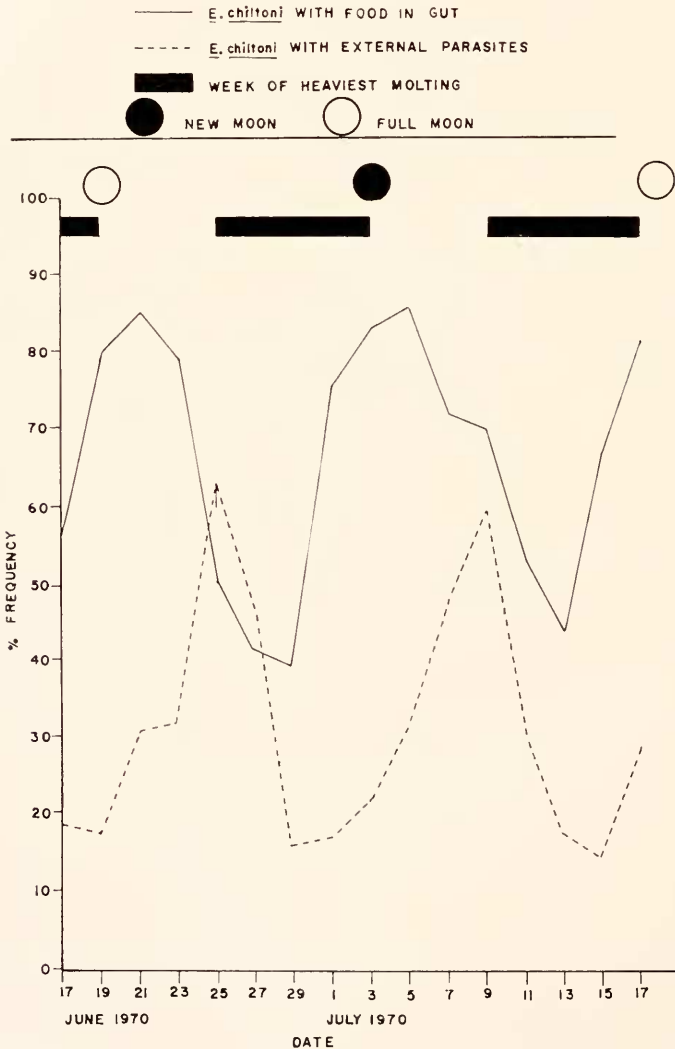


FIGURE 4. Relative abundance of feeding and parasitized isopods as a function of time.

of food in the habitat or there may be innate changes in feeding propensity, associated with the molt cycle. In the first case, periodic feeding could be the cause of molt synchrony; in the second case, it would be an incidental effect.

Fluctuations in the availability of food could arise by either of two processes. There might be a semimonthly variation in the actual concentration of food resources on the beach, or there could be a variable intake of food, due to fortnightly changes in illumination: *Excirolana* forages during the high tide, so that the light regime at which it feeds undergoes a regular fortnightly change as the time of peak tide progresses through the day-night cycle. If visual clues are important for the isopods to locate food items, the illumination cycle could lead to a fortnightly change in feeding activity.

If the first hypothesis is correct, then the percentage of feeding individuals in each of the 5 stages of the molt cycle should show the same fortnightly periodicity. The proportions of feeding individuals in each of the 5 stages should increase simultaneously when food becomes available, and decrease simultaneously when food once again becomes scarce. Kendall's concordance analysis (Table I) was employed to test this hypothesis. The percentage of isopods in each stage of the molt cycle with food in their gut for each of the 16 field collections is given in Table I. The 16 values were ranked across the rows for each stage and the columns of ranks summed. If there were a periodic availability of food, then in some collections all of the 5 stages should simultaneously show elevated feeding levels while in other collections there should be a simultaneous decline. This would lead to a marked departure of individual column rank sums from the average value of all column sums which is 34. In other words, there would be a high degree of correspondence in the percentage of feeding individuals for all stages of the molt cycle on each sampling day, which would lead to a significant probability for the Kendall concordance coefficient (Tate and Clelland, 1957, page 19). This is not the case (Kendall's concordance coefficient = 0.156, $0.90 > P > 0.75$) which suggests that there is no fortnightly cycle in the availability of food.

A runs test for periodicity (Wallis and Roberts, 1956, page 572) applied to each of the intermolt stages (stages 1 through 4) indicates a significant periodicity in the level of feeding activity for the 1st intermolt stage ($P = 0.001$), but not for the 2nd, 3rd, and 4th stages. The periodicity observed for the 1st intermolt stage is, however, a predictable consequence of the fact that it follows the half-molt stage which does not feed. The transition from a non-feeding to feeding condition in the 1st intermolt stage could account for periodicity in its level of feeding activity. The lack of concordance in the level of feeding activity for all stages seems more important for considering the possibility of a periodic availability of food than periodicity in the feeding level of any single stage and indicates that the probability of an isopod obtaining food is independent of whether the animal is molting in phase or out of phase with the rest of the population. Therefore, the availability of food in the habitat does not seem likely as a factor leading to synchronous growth and molting.

The feeding cycle which becomes evident when the entire population is considered (Fig. 4) appears to be the result of a decrease in the propensity of *Excirolana* to feed as it approaches the time of molt. This becomes clear when the average level of feeding activity for the 5 stages of the molt cycle is considered

TABLE I.
Concordance analysis by ranks of the percentage of feeding individuals in each of the 5 stages of the molt cycles;
Kendall's concordance coefficient, $W = 0.156$ ($0.90 > P > 0.75$)

	June										July										Average of 16 values (%)												
	17		19		21		23		25		27		29		1		3		5			7		9		11		13		15		17	
	Rank	% feeding	Rank	% feeding	Rank	% feeding	Rank	% feeding	Rank	% feeding	Rank	% feeding	Rank	% feeding	Rank	% feeding	Rank	% feeding	Rank	% feeding		Rank	% feeding	Rank	% feeding	Rank	% feeding	Rank	% feeding	Rank	% feeding	Rank	% feeding
1st stage (dermoliths absent)	66.1	4	82.3	10	88.4	13	91.2	14.5	75.3	61.4	49.4	91.2	14.5	93.7	16	85.4	11	78.2	9	70.0	66.6	5	58.8	2	76.3	8	87.0	12	76.2				
2nd stage (small demoliths)	100.0	14.5	75.0	1	83.8	4	86.6	7	100.0	100.0	14.5	84.7	6	86.9	8	92.4	11	88.7	10	81.4	100.0	3	78.8	2	96.6	12	89.0						
3rd stage (medium dermoliths)	0.0	1.5	100.0	16	75.0	15	38.4	10	13.2	17.8	0.0	30.0	8	16.0	4	40.0	12.5	39.1	11	63.1	33.3	9	40.0	7	24.0	6	34.1						
4th stage (large dermoliths)	0.0	3.5	0.0	3.5	25.0	15	11.5	7	1.9	3.8	0.0	13.3	14	7.1	3.5	0.0	3.5	16	50.0	10.0	11.5	1.3	4.0	9	0.0	8.5							
5th stage (half-molt)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					
Rank sums	23.5	30.5	48.0	40.0	24.0	30.5	20.5	42.5	38.0	38.0	33.5	39.0	40.0	29.5	36.0	30.5																	

Excluded from analysis since this stage does not feed

(see last column in Table I). There is a clear drop in the level of feeding activity in the 3rd through 5th molt stages. The decrease in the percentage of feeding individuals in Figure 4 occurs about the time that these stages begin to make up a significant proportion of the population (see Fig. 3). In summary, the fortnightly feeding cycle appears to be an effect rather than the cause of synchronous molting.

The apparent synchronization of molting activity to the spring-neap cycle could perhaps be the result of a synchronous input of young. These might then develop at approximately the same rate and, at least initially, molt in synchrony. One would expect that differences in the growth rate of individual isopods should eventually lead to a deterioration of synchrony after several molts.

To examine this possibility, the frequency of occurrence of the 1st intermolt stage (isopods which lack dermoliths) was computed separately for the 1st manca, 2nd manca, 3rd manca and post-manca males and females. This single index of the molt cycle was chosen because it is, on the average, better represented in the samples than the other four stages. Estimates of the abundance of this stage are, therefore, subject to a smaller degree of sampling variability than the other less abundant stages. These data are shown in Figure 5. Note that molting for each of the developmental stages is synchronous and phased to the tides. There is no apparent loss in synchrony, even in the post-manca males and females. These latter stages have undergone at least 3 molts in the field. In fact, some individuals in the post-manca categories were born in the previous year (Klapow, 1971), and judging from their size had probably undergone several additional molts since birth. It appears, then, that development is continuously adjusted so that molting will at all times occur at the same phase of the spring-neap cycle.

This does not exclude the possibility of a synchronous input of young. Such a mechanism could account for initial molt synchrony but would not provide an adequate explanation for the maintenance of synchrony over several molts. A most interesting aspect of these data is, in fact, the apparent synchronization of the first molt following birth. The 1st stage mancas molt in phase with later developmental stages. This would suggest the possibility that the 1st stage mancas might have been born in synchrony.

Further evidence which supports the proposition that 1st stage mancas molt in synchrony is presented in Figure 6 (graph A). Graph A is a plot of the relative abundance of half-molt individuals in transition from the 1st to 2nd manca stage, expressed as a percentage of the 1st stage mancas. Synchronization is apparent. Most of the molting activity takes place on the weeks prior to new or full moon, as is the case in later developmental stages. If the 1st stage mancas were born continuously, there would have to be considerable variation in the duration of the intermolt in order to achieve synchrony in molting. If, on the other hand, the delivery of young as well as molting, were phased to the tides, this problem would not arise.

There is no way of identifying a newly released isopod with complete certainty. One might however expect a newborn individual to have the following characteristics. It would, of course, have to be a 1st stage manca. In addition it might not have been in the environment long enough to have fed or acquired external parasites. While dermoliths are present in the late embryonic stages, one might expect

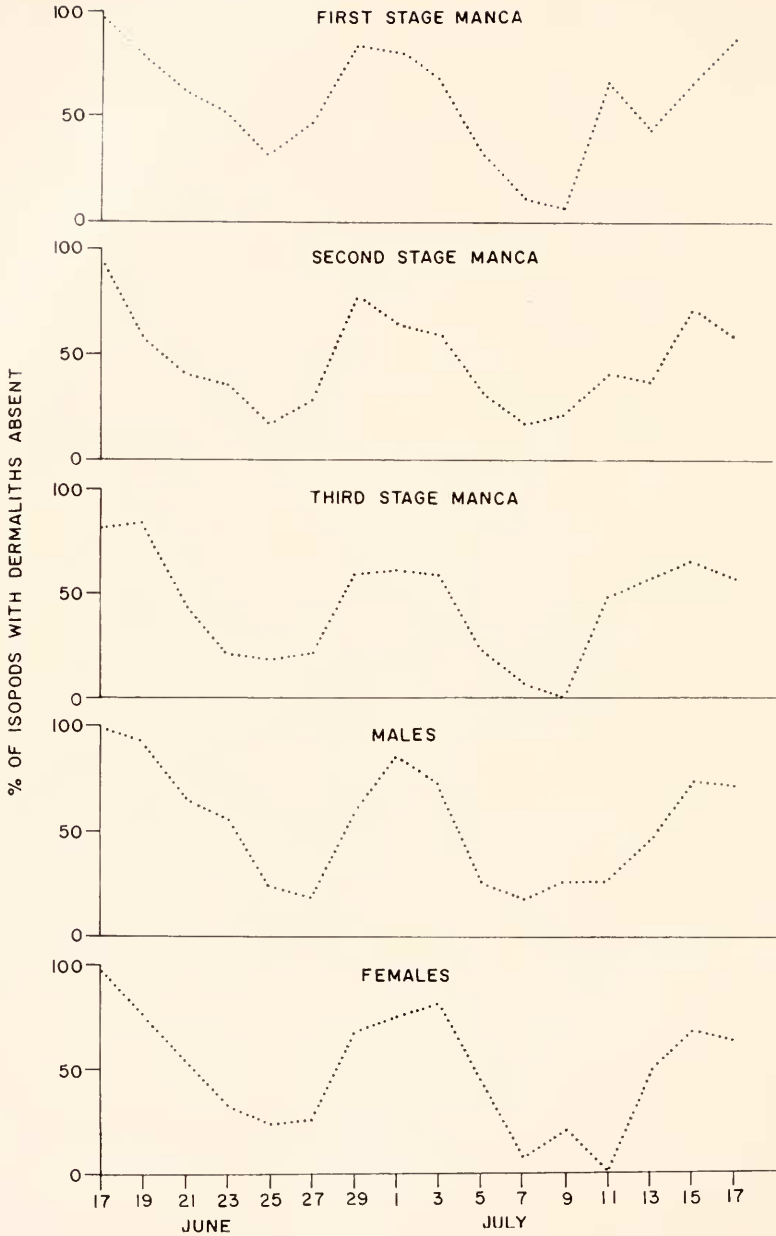


FIGURE 5. Relative abundance of isopods in the 1st intermolt stage plotted separately for developmental stages.

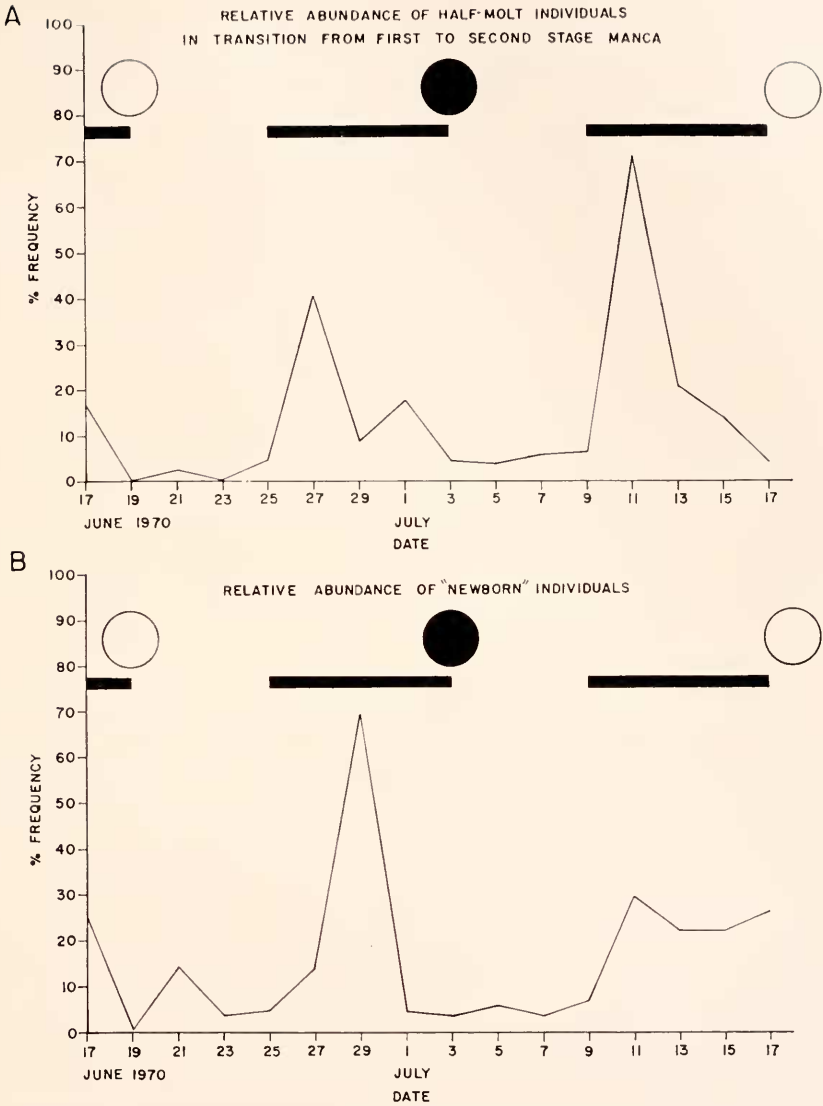


FIGURE 6. Synchronized molting and birth of 1st stage mancas. Open and closed circles indicate the day of new and full moon respectively. Black bars indicate the period of heaviest molting activity.

that they would be rapidly absorbed as the soft integument expands and hardens following birth. Dermal molts are completely absorbed in less than 25 hours in the half-molt stage and assuming that this rate of absorption applies to newly released isopods, they would then lack dermal molts shortly after birth. These three characteristics, the absence of dermal molts, of external parasites, and of food in the gut were employed to identify 1st stage mancas which were likely to have been recently

released into the environment. Figure 6 (graph B) shows the relative abundance to these "newborn" individuals expressed as a percentage of all 1st stage manca. If one accepts the criteria for identifying newborn isopods, it is quite clear that the release of young was synchronous and occurred at the same phase of the spring-neap cycle as molting in the population. An extremely well synchronized release of young occurred between June 27 and July 1 (Fig. 6, graph B) which presumably involved the same individuals which showed the highly synchronized molting activity which occurred approximately a fortnight later on July 9 through July 13 (Fig. 6, graph A). Thus newborn individuals are apparently released synchronously and molt two weeks later at the same phase of the tides during which they were born.

Molting in the laboratory

On June 23, 1970, 60 juvenile (manca stage) isopods were brought in from the field and their molting activities were monitored for 3 months. Each isopod was placed in a 50 ml vial which was filled with sand to a depth of 1 cm. A mesh

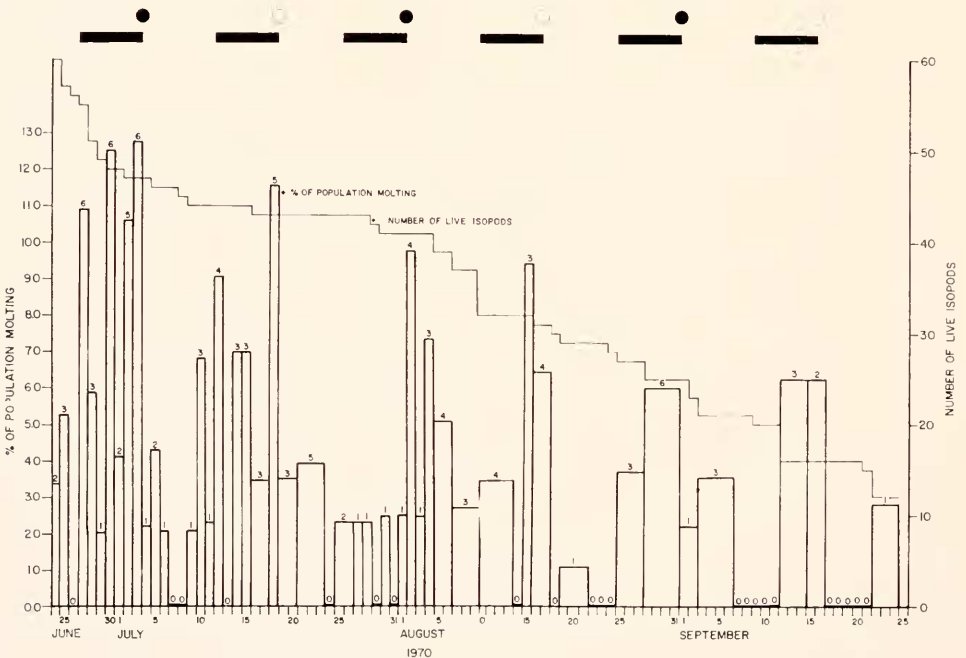


FIGURE 7. Molting frequency of *Excirolana chiltoni* in the laboratory. Molting frequency is indicated by the number of posterior casts recovered on the day of examination (number above each bar) which is expressed as a percentage of the isopods that were alive at that time. All mortality was assumed to have taken place on the first day following the last examination when the interval between examinations was greater than a day. When the time between examinations was greater than a single day the average daily molt frequency for that interval is shown. Open and filled circles indicate the days of full and new moon, respectively. Bars represent the expected time of heaviest molting activity in the field.

window was inserted in the vial above the sand level to allow water to circulate through the vial when it was placed on a water table in Scripps Aquarium. Light was kept at a constant dim level but no attempt was made to control temperature which varied considerably over the study interval (17.5° C to 22.8° C). Each vial was stocked at regular intervals with an excess of food in the form of pieces of *Thoracophelia mucronata*, a major component of *Excirolana*'s diet on Scripps Beach. The vials were examined for the presence of cast exoskeletons every day for the first 21 days of the study and at 1 to 4 day intervals thereafter, at irregular times of day.

Considerable mortality occurred during the course of this molting study; molting frequency for the population is therefore expressed as a percentage of live

TABLE II.

Molt intervals for Excirolana chiltoni in the laboratory (all entries are in days)

	2nd manca	3rd manca	1st post-manca	2nd post-manca
	26	25	21	30
	19	26	30	24
	20	17	23	42
	18	18	22	39
	25	17	32	39
	17	16	20	52
	19	25	24	29
	17	20	29	33
	19	24	23	29
	15	18	22	32
	17	19	26	24
	20	21	27	
		22	22	
		27	30	
		32	39	
			30	
			31	
			41	
			23	
			27	
			30	
Range	15-26	17-32	20-41	24-52
Mean interval	19.33	21.80	27.5	33.91
95% C.L. on mean interval	17.28-21.38	19.27-24.33	25.06-29.93	28.24-39.57

isopods on the day of observation (Fig. 7). A weak periodicity is apparent for the first few weeks of the study. Molting was common during the week prior to the new moon of July 3, declined during the following week and rose again on the week prior to the full moon of July 18. Beyond the first cycle, the times at which the population was checked were too irregular to permit firm conclusions about the maintenance of synchrony. The suggestion of continued synchrony (note that high values usually occurred on days of full and new moon) may be only coincidental.

Table II lists the duration of developmental stages in the laboratory. Only those intervals which were known to an accuracy of one day are recorded. These

data differ from field observations in two respects: the duration of each stage is significantly greater than the semimonthly tidal interval of 14.76 days; and there is a progressive lengthening of the duration of each instar from an average of 19.33 days for the 2nd manca to 33.91 days for the 2nd post-manca.

In the field, all instars molt at the same phase of the spring-neap cycle (Fig. 5) which means that the only molt intervals realized under field conditions must be either 14.76 days or some whole multiple of 14.76 days. If there was a strong endogenous rhythm controlling the molting cycle, one would expect that the duration of instars under non-tidal conditions in the laboratory would be a whole mul-

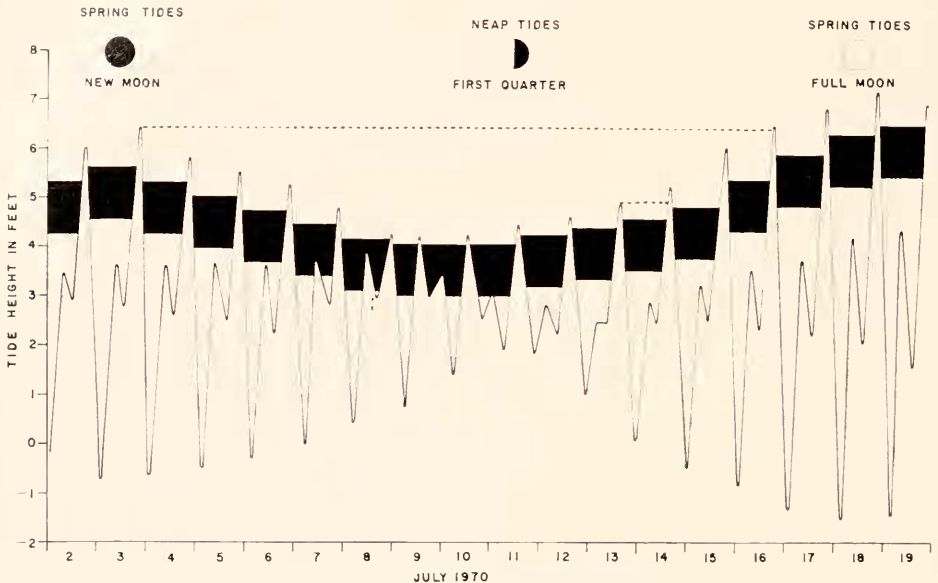


FIGURE 8. Idealized representation of the fortnightly movements of the isopod zone. Tide data are real but the position of the isopod zone is hypothetical. Blackened bars represent the position of the isopod band with reference to tide level (see Fig. 1 for field data substantiating the existence of the fortnightly movement of the zonal band). Dotted lines illustrate the fate of isopods which are stranded at the edge of the wave wash at high tide. Isopods stranded during the transition from spring to neap tides might be marooned above the water line for as long as 2 weeks, while animals stranded during the transitions from neap to spring tides would be reached by the high tide on the following day.

tle of some basic period which approximates, though need not be precisely, 14.76 days. This was not observed: the duration of consecutive instars increased progressively and there were no clear modes at or near multiples of 14.76 days.

DISCUSSION

Excirolana shows fortnightly rhythmicity not only in the release of the young, but also in locomotor activity (Heisner and Enright, 1966), feeding, molting and the extent of parasitism. The question therefore arises: which of these, if any, is

the primary rhythmicity, and which of these rhythms is of major ecological advantage to the organism? In brief, what is the adaptive significance of these phenomena?

Fortnightly cycles which have been previously studied deal primarily with reproduction. Synchronization of reproduction to the lunar cycle is particularly common among species with external fertilization which shed their gametes free into the environment. The first well documented example of lunar spawning and still one of the most dramatic cases is found in the Palolo worm, *Eunice viridis*, of the South Pacific Islands. Field observations on lunar spawning cycles are by no means confined to marine annelids, although these make up the bulk of well documented cases in the older literature. Korringa (1947, 1957), in his extensive surveys of the literature, found evidence for lunar spawning cycles in marine coelenterates, echinoderms, crustaceans, insects, and vertebrates. His reviews, while comprehensive in scope, did not involve critical evaluation of the claims, so that while there is reason to believe that lunar reproductive cycles are widespread, there are still only a few well established cases. One of these is the spawning cycle of *Leuresthes tenuis*, a small offshore fish which runs up on the beaches of Southern California and Mexico to spawn shortly after tide crest, over a period of 3 or 4 days beginning a day or two after new or full moon (Walker, 1949). The adaptive value of reproductive synchrony is obvious for species with external fertilization: by restricting the liberation of gametes to a particular time and place, the probability of successful fertilization is much enhanced.

Synchronous mating can also be beneficial for species with internal fertilization where physical contact between the sexes is required. In many crustaceans, impregnation of the female is only possible during a short interval which follows the molt. In the marine isopods, *Idotea emarginata* (Naylor, 1968), and *Idotea neglecta* (Kjemmerud, 1950) insemination takes place as the female molts, as is the case with *Excirolana*. The availability of many receptive females, as a consequence of the synchronized molt, might enhance the incidence of successful matings by increasing the sexual responsiveness of males at this time. It is generally recognized that for those species where sexual receptiveness is intermittent, an appropriate state of behavioral and physiological readiness must be simultaneously achieved in both sexes if successful reproduction is to follow. Wheeler's (1937) study of the shrimp *Anchistioides antiquensis*, is directly pertinent for considering the possibility of synchronous reproductive activity in *Excirolana*. He found that this prawn swarms at the surface in the field on nights following the new moon. In addition he found what may have been a lunar rhythm in molt frequency in the laboratory. His data on molting are not very extensive (only a single cycle was observed) and the supposed lunar cycle is not particularly clear, but the possibility exists that there might be a causal relation between swarming in the field and the molting cycle which Wheeler observed in the laboratory, although he did not believe that this was the case.

Synchronous mating can convey other benefits in addition to increasing the likelihood of successful contacts between the sexes. Synchronous mating in some instances is required to facilitate the development of embryonic or juvenile stages in coordination with the tides so that their liberation will occur at a time when environmental conditions are most suitable. According to Hagen (1970) the

necessity of releasing planktonic larvae at spring tide has apparently led to synchronous courtship and mating in the fiddler crabs *Uca annulipes*, and *Uca triangularis*. Courtship displays are most intense a few days before new or full moon, which is followed by insemination around the time of new or full moon and the planktonic larvae are released two weeks later on the spring tides. In the grunion *Leuresthes tenuis*, eggs which are deposited on the high beach during spring tides are incubated in the warm sand for approximately two weeks. The larval fish emerge from the egg capsule when they are wet by the spring tides of the next cycle. In all of the above cases the release of young takes place a fortnight after fertilization. *Excirolana chiltoni*, however, incubates its brood for approximately 2 to 3 months. The need for synchronous initiation of development is, therefore, not immediately apparent since the release of young at a particular phase of the spring-neap cycle could be accomplished by adjusting the duration of embryonic development by only a few days, that is, by a small fraction of the total gestation period. The synchronous molting and insemination of mature females which might possibly aid in achieving a synchronous release of young 2 to 3 months later, does not account for the fact that pre-reproductive isopods molt in synchrony as well.

Both of the hypotheses suggested up to this point have dealt with only a part of the total phenomena. An adequate hypothesis would have to account for the following facts: the release of young, as well as the molting of every developmental stage, is synchronized to the spring-neap cycle, and the phase of these activities corresponds to the transition from neap to spring tides (a time when the amplitude of the tide is increasing on each consecutive day). Figure 8 illustrates a hypothesis which provides a unified basis for interpreting the ecological significance for both the synchrony and phase of these processes. *Excirolana chiltoni* maintains a relatively constant position on the beach with reference to the high tide wash line over the spring-neap cycle. Isopods burrow down into the sand and escape the falling tide at a fixed time past tide crest (Klapow, 1971). This behavior will assure that *Excirolana* is washed at high water at least once each day. Two requirements would seem essential for maintaining this distribution. The first would be a precise sense of timing to allow the isopods to escape the receding tide at a level of the beach which will be reached by the next period of high water (Klapow, 1972). A second requirement would seem to be the continuous maintenance of swimming and burrowing ability since an incapacitated isopod might be carried to the limits of the wave wash in the same fashion that debris is washed up on the beach. Chandrashekaren (1965) observed that the mole crab, *Emerita asiatica*, when in a quiescent state following the molt, is carried up the beach to the limits of the wave wash. There is no reason to suspect that this would not happen to *Excirolana chiltoni* as well.

If an isopod were stranded at the upper limits of the wave wash at peak tide, its fate would depend to a great extent on when this happened in the spring-neap cycle. If it were stranded at peak tide during the transition from spring to neap tides, it could be marooned on the high beach, above the reach of the wave wash, for as long as two weeks. If, on the other hand, this were to happen during the transition from neap to spring tides, the consequences would be far less severe. An individual stranded at the upper limit of the wave wash at this time would be

reached by the high tide on the following day and could reestablish an appropriate position in its habitat on the very next cycle.

The principal assumption underlying this hypothesis is that *Excirolana* is less able to maintain position in the wave wash during its molt, because of reduced swimming and/or burrowing ability. One expectation deriving from this interpretation is that molting individuals might have a distribution higher on the beach than nonmolting individuals. The field data were examined for indications of such a trend. In those cases in which statistically significant differences in distribution were present, molting individuals were, indeed, somewhat farther landward than nonmolting, but in most sampling series, no significant differences in the distributions of these two classes were detectable. Hence, the data do not provide strong support for the proposed interpretation.

Other data (Klapow, 1971) indicate that heavy storm waves can occasionally lead to major mortality in the population, and it is primarily at such times that molting individuals would be expected to be particularly vulnerable. During the present sampling program, surf conditions were rather calm (average swell of 1.9 feet); hence, the lack of strong support for the stranding hypothesis in the available data may indicate only that the synchronization of molting is an adaptation to sporadically occurring conditions which did not prevail during the sampling interval.

In summary, the stranding hypothesis, although not completely substantiated by the data, provides a unifying framework for the interpretation of *Excirolana's* fortnightly rhythms. The ecological advantage in synchronizing molting to the rising series of tides is clear and would seem to be the primary adaptation; other fortnightly rhythms appear to arise primarily as a means of achieving molt synchrony or merely as incidental consequences of the molt cycle. For example, the synchronous release of young facilitates synchronization of the first molt following birth to the appropriate phase of the tidal cycle while the other rhythms (*i.e.* feeding and parasitism) appear to be incidental consequences of the molt cycle.

The possibility exists that synchronous birth might constitute an adaptation in its own right, since newborn isopods with soft exoskeletons might also be subject to a high risk of stranding. While the data which suggest that the release of young at a particular phase of the spring-neap cycle (Fig. 6) involve indirect evidence, this conclusion is rendered more plausible by the unique reproductive morphology of this species which allows it to control the time of birth. In typical free-living isopods, the young are brooded in an external pouch, out of which they can crawl or swim after they have reached an appropriate stage of maturity. In contrast, *Excirolana chiltoni* is ovoviviparous (Klapow, 1970); the female retains her embryos within sacs which are completely sealed off from the external environment up to the time of birth. The young cannot escape until external openings to the chambers in which they lie develop on the ventral surface of the gravid female. Even after the development of these openings the embryos are not free to leave. Birth is apparently accomplished by an increase in the fluid volume of the female's hemocoel, which exerts pressure on the sacs in which the embryos lie, causing them to contract and eventually evert through the openings. The timing of birth is, therefore, activated by physiological processes occurring in the gravid female.

The storage of calcium reserves has obvious benefits for terrestrial and fresh water crustaceans, where this element is often depleted in the environment. It is, however, more difficult to develop a similar argument for the storage of calcium reserves in marine forms, since calcium is a major component of the dissolved salts in sea water (420 mg per liter). Therefore, one would not expect that calcification of the post-ecdysial integument to depend very heavily on an internal supply. In fact, the major part of the integumental calcium of marine decapods is absorbed from the external medium following ecdysis (Drach, 1939; Hecht, 1914; Needham, 1954; Robertson, 1937; Travis, 1955; Lafon, 1948).

It has, however, been reported by a number of authors that prior storage of mineral reserves leads to a more rapid hardening of the exoskeleton following ecdysis, and perhaps this is the major ecological advantage for storing mineral reserves in *Excirolana*. Paul and Sharpe (1916) found that of the three genera of decapods which they studied (*Cancer*, *Lithoides* and *Homarus*), *Cancer pagurus* stores concretions of calcium phosphates in the hepatopancreas to the highest degree and as a consequence post-ecdysial hardening of the exoskeleton is more rapid than in the other species studied. An interesting observation bearing on this point was made by Numanoi (1939) in his study of the shore crabs *Sesarma haematocheir* and *Sesarma dehaani*. The two species differ in that *S. haematocheir* produces gastroliths (concretions of calcium carbonate formed by the gastric epithelium) prior to molting while *S. dehaani* does not. Both species seek refuge in shallow pools at the time of ecdysis; *S. haematocheir* takes only two days to complete hardening of the exoskeleton, while *S. dehaani* requires nearly twice as long. Two lines of evidence support the idea that stranding on the high beach poses a potential hazard for *Excirolana*: (1) the synchronization of molting and birth to the weeks of rising high tides and (2) the storage of mineral reserves which presumably decrease the interval of reduced locomotor function following the molt.

The fact that a fortnightly molting rhythm was not particularly evident under non-tidal conditions in the laboratory argues against an endogenous controlling mechanism but does not completely exclude this possibility. It is generally recognized that the persistence of endogenous rhythms under constant conditions depends to a great extent on the particular conditions under which the organisms are kept (Bünning, 1967). The considerable mortality of isopods during the study (Fig. 7) suggests that the conditions to which they were subjected were less than ideal. The major source of mortality during the study resulted from clogging of the mesh screens which restricted the circulation of water through the vials. The high degree of variability in the duration of instars (see ranges in Table II) as well as the longer than fortnightly interval between molts might be artifacts of the experimental conditions in which stresses imposed by restricted circulation through the small vials and the availability of only a single food item (*Thoracophelia mucronata*) might have interfered with the normal developmental processes. *Excirolana chiltoni* shows clear monthly and semimonthly rhythms in swimming activity under constant laboratory conditions which persist for several cycles when the animals are kept in large containers (Heusner and Enright, 1966; Klapow, 1971; Enright, 1972) so that an endogenous timing mechanism with an appropriate period is at least available to this species. Furthermore, the

fact that under field conditions the accumulation of mineral reserves takes place in anticipation of the actual molt (Fig. 3) indicates that at least some measure of internal timing is involved. Nevertheless, the possibility of exogenous influences cannot be excluded; perhaps periodic exposure on the high beach is necessary to maintain and accelerate the molt cycle.

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SUMMARY

1. The isopod, *Excirolana chiltoni* is found buried in the sand in a narrow zonal band on the high intertidal beach during low tide and emerges only during high tide to swim and forage in the wave wash. The position of the zonal band during low tides bears a constant relationship to the level of the water line during the preceding high tide. Because of this relationship the isopod zone moves up and down the beach on a fortnightly schedule which corresponds to the semi-monthly variation in tide amplitudes (the spring-neap cycle).

2. *Excirolana chiltoni* stores considerable quantities of mineral prior to ecdysis in localized concretions of the integument (dermoliths).

3. Molting takes place in two steps. First the posterior portion of the isopod including the abdomen and last 3 thoracic segments is cast which is followed by the casting of the anterior portion of the animal. Isopods which are in the process of molting can be easily recognized since the newly exposed posterior exoskeleton is fully expanded before the anterior cast is made.

4. The development of dermoliths and the relative abundance of half-molted individuals were used to determine the frequency of molting in a field population which was sampled at 2 day intervals for a period of a month. These data indicate that molting is largely if not entirely restricted to the weeks preceding new or full moon when the amplitude of the tides is increasing.

5. *Excirolana chiltoni* also shows fortnightly periodicities in feeding and the degree to which it is infested with external parasites.

6. Indirect evidence suggests that the release of young also follows a fortnightly schedule and is most intense at the same phase of the spring-neap cycle that the population molts. Females retain their broods internally and might therefore be capable of controlling the timing of birth with considerable precision.

7. Synchronous molting was not as apparent in populations which were held under non-tidal conditions in the laboratory.

8. Synchronization of molting and birth to the ascending series of high tides and the storage of mineral reserves prior to ecdysis are interpreted as adaptations which serve to decrease the probability of an isopod being marooned above the waterline for extended periods of time where it would be subject to a high risk of death by desiccation.

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